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BODY FLUID CONCENTRATIONS OF NICOTINE AND COTININE AFTER CONTROLLED EXPOSURES TO ENVIRONMENTAL TOBACCO SMOKE

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ABSTRACT

Salivary and urinary concentrations of nicotine and cotinine were measured in 17 nonsmokers before and after exposure to ETS nicotine (range, 1 - 54 $\mu g/m^3$) in a controlled environmental room (CER). Biomarker concentrations were determined in saliva pre-exposure and immediately, 3.5 and 19.5 hrs post-exposure, and in 24-hr urine at 24 and 48 hours. Salivary nicotine, sampled immediately post-session and adjusted for pre-session body burden, was most closely correlated to nicotine exposure. Salivary cotinine concentrations were related to nicotine intake at each post-session sampling interval. Urinary biomarkers indicated that nicotine exposure occurred, but no dose-response relationship was discerned.

INTRODUCTION

Nicotine and cotinine have been evaluated as biomarkers of environmental tobacco smoke (ETS) by an increasing number of investigators in recent years. Neither candidate meets all the National Academy of Science criteria (1), however, and field studies have not provided evidence that degree of ETS exposure can be predicted reliably from the amounts or concentrations of cotinine or nicotine in body fluids (2, 3). As part of our efforts to quantify potential short-term effects of ETS on nonsmokers (4, 5), we conducted a controlled exposure study to assess sensory and psycho-physiological responses to ETS. This involved exposing 17 nonsmoking male subjects, over the course of multiple test sessions, to a control and five ETS levels that ranged from approximately 4 to 60-fold those observed in actual indoor environments in the US where smoking is unrestricted (6).

This study afforded an opportunity to evaluate salivary and urinary nicotine and cotinine as biomarkers of ETS exposure under tightly controlled conditions in which many of the difficulties inevitably present in field studies could be minimized or eliminated. Each subject served as his own control, the duration of ETS exposure and its timing with body fluid collection were held constant, and we attempted to minimize exposure before and following each test session. Thus our results provide some indication of the "best case" performance of these candidate biomarkers under idealized conditions.

METHODS

Subjects: Twenty (20) subjects were recruited by a marketing research firm and satisfied the initial entry requirements: normal spirometry, salivary cotinine concentration < 10 ng/mL, and completion of an Informed Consent Form that certified: a) their state of health, b) lack of use of contact lenses, and c) no association with the tobacco industry. Subjects' mean

RJW data not shown.

age, height, and weight (± SEM) were 27 ± 0.99 years, 177 ± 1.07 cm, and 83 ± 2.06 kg, respectively.

Controlled Environment Room: The controlled environmental room (CER) has a volume of 45 m^3. Air supplied to the room was dehumidified, charcoal- and HEPA-filtered, then reconditioned to maintain a constant temperature (22 °C) and relative humidity (50%).

Chemical Measurements: Time-weighted average measurements of nicotine were determined by methods described by Ogden (7). Additional ETS analytes to characterize the CER atmosphere are described elsewhere in these *Proceedings* (6).

Experimental Design: Subjects were tested in pairs, except for three individuals whose partner dropped out, under six conditions consisting of a clean-air Control and five levels of ETS. Subject pairs were exposed multiple times to each condition.

Exposure Session: Subjects were instructed to avoid ETS exposure for 48 hours before and after each session and to record in a daily diary any known exposure to ETS. Before entry into the CER, subjects provided a saliva sample and a spot urine sample. Respiratory measurements were taken via a calibrated Respicomp™ system (Respitrace Corp., Ardsley, NY) after subjects were seated in the CER. Breathing data for each subject were collected into a file for each session. Each CER exposure session was 90 minutes with ETS generated for 70 minutes by four smokers separated from the subjects by a partition. A time scheme for ETS nicotine exposure and body fluid samples is shown in Figure 1. Subjects maintained body fluid samples at 4 °C in an insulated container with artificial icepacks until return after the 48-hr urine sample. Body fluid samples were processed and frozen at -20 °C until analyzed for nicotine and cotinine.

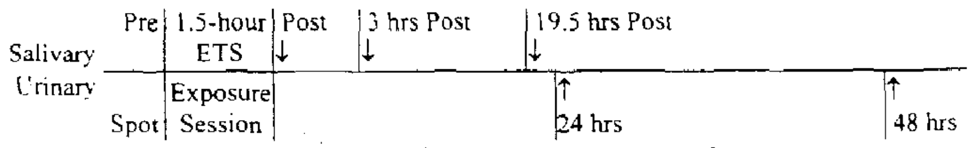

Figure 1. Time Points for Salivary (↓) and Urinary Samples (↑): The timing of ETS nicotine exposure and body fluid sampling for each 'subject × session' CER session is shown. Each session was 1.5 hrs with 70 minutes of ETS exposure.

Biomarker Analyses: Salivary nicotine and cotinine were determined by radioimmunoassay (RIA) as described (8). Urinary nicotine and cotinine were determined by capillary gas chromatography with nitrogen-specific detection by a contract laboratory. The experimental design for ETS exposure of subjects in the CER was based on achieving specific RSP levels provided by four smokers within an experimental session. More germane to body fluid measurement of nicotine and cotinine is the nicotine intake per subject per session, determined by the equation:

$$\textit{Nicotine Intake}, \mu g = \textit{Time-weighted air [nicotine]}, \mu g/m^3 \times \textit{Breathing volume}, m^3$$

Statistical Treatment: Both salivary and urinary data sets consisted of 275 records of 'subject × RSP' data. Due to malfunction of real-time analyzers or failure to meet the exposure criteria for a particular session, subjects were not exposed equally often to each RSP condition. Therefore, replicates of each 'subject × RSP' combination were averaged to yield 17×6=102 records for subsequent analysis (i.e., one record for each subject at each RSP condition). A single salivary record consisted of four values each for salivary nicotine and co-

tinine, CER air nicotine measurement, subject's breathing volume, and subject and RSP identifying variables. A single urinary record consisted of three values each for urinary nicotine and cotinine, urinary creatinine, urine volume and pH, the CER air nicotine measurement, the subject breathing volume, and subject and RSP identifying variables. Nonparametric analyses were required due to heterogeneity of variance across design levels. Using design RSP levels as a grouping variable, and matching on subject when appropriate, these nonparametric statistical analyses were done: a) Kruskal-Wallis test for equality of populations, b) Cuzick's test for trend across ordered groups, and c) pairwise one-sided matched-sample sign tests for equality of medians.

RESULTS

Although subjects were instructed to avoid ETS exposure for 48 hours before and following the exposure sessions, we observed some degree of non-compliance for approximately two-thirds of the subjects. Therefore, half-life estimates were used to partially adjust the post-session salivary biomarker data for the pre-session body burden. Half-life was determined by regressing biomarker loss data against the pre-exposure body burden to yield estimates for the average loss proportion (as the slope) and for the increase due to the residual nicotine dose (as the intercept). Biomarker loss itself was determined by taking the difference of pre- and post-exposure concentrations of salivary nicotine and cotinine for Control sessions. This yielded an estimate of loss during a defined time interval (2 hours) where exposure to nicotine was essentially trivial (mean = 1.15 $\mu g/m^3$, due to residual nicotine). Half-life, in hours, is then computable as:

$$Half\text{-}life = 2\ hrs / \left(\log(1 - loss\ proportion)\ \log(0.5)\right)$$

The half-life estimate for nicotine was 1.1 hrs (95 % CI: 0.8 to 1.5 hrs). For cotinine the half-life estimate was 17 hrs (95% CI: 11 to 39 hrs). Exposure to the residual nicotine (1.15 $\mu g/m^3$) was estimated to increase the salivary nicotine concentration by 1 ng/mL, but had only a negligible, non-significant effect on cotinine. The half-life estimates for salivary nicotine and cotinine were used to adjust the salivary data at each post-exposure sample time. That is, the concentrations of salivary nicotine and cotinine due to designed ETS exposures were estimated to be the observed post-exposure concentrations minus the fraction of pre-exposure concentration remaining at that time point.

Table 1. Environmental Room and Subject Characteristics: Tabled entries are median values for each designed ETS-RSP condition.

Design ETS-RSP, $\mu g/m^3$	0	50	100	200	400	800
Observed ETS-RSP, $\mu g/m^3$	0	58	113	217	368	765
Air Nicotine, $\mu g/m^3$	1.3	13.4	19.6	23.4	26.9	53.8
Breath Volume, m^3	0.810	0.827	0.781	0.822	0.816	0.777
Nicotine Intake, μg	1.0	11.2	16.2	19.7	21.2	42.5

Table 1 reports the design ETS-RSP levels and the observed ETS-RSP levels achieved by four smokers generating *true* ETS within the CER. Observed ETS-RSP levels approximated design ETS-RSP levels, but air nicotine concentrations did not parallel the ETS-RSP

levels. Consequently, median nicotine intake amounts tend to nearly plateau at the intermediate ETS-RSP levels. Nonetheless, adjusted salivary nicotine concentrations measured immediately post-exposure showed statistical evidence of overall significant differences ($p<0.001$) that had an increasing nonparametric linear trend component associated with ETS exposure levels ($p<0.001$). No evidence of the designed ETS exposures could be detected by salivary nicotine at either 3 or 19.5 hrs post-exposure. These results are consistent with the half-life of nicotine: less than 12% of the designed nicotine exposure would remain at 3 hrs post-exposure; only 0.001% would remain at 19.5 hrs.

Salivary cotinine exhibited some statistical evidence of overall significant differences for samples obtained immediately post-exposure ($p<0.001$) and for samples obtained at 3 hrs post-exposure ($p<0.001$), but not for samples at 19.5 hrs ($p=0.176$). An increasing nonparametric linear trend component was significant at all three post-exposure time periods ($p<0.001$, $p<0.001$, $p=0.006$). All of these statistical analyses were dominated by the difference of the highest exposure level from all other exposure levels. No effect or trend was discernible among the five lower levels.

Urinary nicotine and cotinine concentrations were normalized by the creatinine data, but were not adjusted for initial body burden. The statistical analyses performed were similar to those applied to salivary data. Neither urinary nicotine nor cotinine concentrations, adjusted for creatinine, exhibited statistical differences due to ETS exposure levels for either the first 24-hr urine sample or the second, 24 to 48-hr sample (all p-values > 0.5). Likewise, no linear trend component was found for either nicotine or cotinine (all p-values > 0.1).

Correlations of nicotine intake to salivary and urinary biomarker concentrations provided a means for ranking biomarker sensitivity (Table 2). Salivary nicotine, an indicator of acute ETS nicotine exposure, measured immediately post-exposure yields a significant correlation ($r=0.98$, $p\leq 0.05$). Due to its short half-life, salivary nicotine at prolonged sampling times showed no relationship to nicotine intake. Salivary cotinine was significantly related to the nicotine intake at all post-session sampling times; this is consistent with the longer half-life of cotinine. The significant correlation of nicotine intake to salivary cotinine measured immediately post exposure was unexpected, but agrees with previously reported data (10).

Table 2. Correlation of Nicotine Intake to Biomarker Levels: Columns marked "n=6" are correlations of the six condition-level nicotine intake and biomarker *median* values. Columns marked "n=102" are correlations of the 102 subject × condition-level nicotine intake and biomarker *mean* values.

Sampling Time	**Nicotine**		**Cotinine**	
Salivary †	**n=6**	**n=102**	**n=6**	**n=102**
Prior to exposure	-0.15	0.01	-0.37	-0.05
Immediately post-exposure	0.98 §	0.69 §	0.93 §	0.43 §
3 hours post-exposure	-0.13	-0.05	0.96 §	0.28 §
19.5 hours post-exposure	-0.68	-0.05	0.82 §	0.10
Urinary ‡				
Pre-exposure (spot sample)	-0.01	0.11	0.15	-0.01
0-24 hours post-exposure	0.84 §	0.15	0.68	0.12
24-48 hours post-exposure	0.29	-0.05	-0.38	-0.07

† ng/mL, adjusted for pre-exposure salivary concentration; ‡ ng/mg Creatinine, 24-hour urine sample; § significant at p=0.05.

Data generated by this investigation allows for the estimation of the minimal amount of nicotine that must be inhaled over 70 minutes for reliable, statistically significant detection as a biomarker in saliva and/or urine. A log-linear model of nicotine intake and salivary nicotine adjusted for pre-exposure body burden was fit using regression. The regression indicates that a nicotine intake of 4 μg must occur for salivary nicotine to be reliably detected by this pool of subjects (n=17). An intake of 30 μg is required for reliable detection by a single subject. Likewise, for salivary cotinine the estimated nicotine intake for reliable detection is 8.5 μg for the pooled subjects and 65 μg for a single subject. Model for urinary nicotine and cotinine indicates that the minimum detectable nicotine intake would be about 17 μg for the subject pool; estimates for a single subject are outside the range of the experiment.

DISCUSSION

Salivary and urinary nicotine and cotinine concentrations provide advantages over blood-borne biomarkers. Subjects can provide both saliva and urine specimens on demand without the discomfort or expense associated with invasive techniques. Both nicotine and cotinine concentrate from the systemic circulation into saliva, thus allowing their quantitation in the absence of quantifiable concentrations in the blood (9). Nicotine concentrates at much higher ratios (0 to 60 times) than does cotinine (~30% higher) (10). Urine provides time-integrated samples for biomarker determinations of nicotine and cotinine. A previous study (9), which exposed subjects to extremely high sidestream smoke levels, suggested that urinary cotinine was best for discriminating nicotine exposure. An ETS-specific study (11), which had excellent control of *ad hoc* exposures, found salivary nicotine, urinary nicotine and urinary cotinine to be discriminators of nicotine exposures. Under our experimental conditions, we found that the salivary biomarker concentrations provided better discrimination of nicotine exposure both from an acute, short-term (salivary nicotine) period and a more prolonged time interval (salivary cotinine).

Deficiencies in the majority of field studies are apparent: a) they either fail to monitor the ambient atmosphere or the individual's exposure to ETS markers, and b) they depend on subjective information regarding time and degree of ETS exposure. In the present study the ETS exposure was due to *true* ETS composed of exhaled mainstream smoke and sidestream smoke from cigarettes provided by four smokers within the CER. Design RSP target levels were obtained by manipulation of the ventilation rate of the CER and the number of cigarettes smoked. The agreement of the measured ETS-RSP levels and the design target levels is indicative that the generation of the ETS in this manner was successful. Air nicotine levels, though, did not exhibit colinearity with the ETS-RSP. Nonetheless, this anomaly of air nicotine has no impact on the biomarker expression of ETS exposure in the subjects. Time-weighted average air nicotine measurements and respiratory measurement allowed the calculation of nicotine intake by subjects during an experimental session. Difficulties arising from the non-compliance of subjects were overcome by adjustment of salivary nicotine and cotinine concentrations for pre-exposure body burdens of the analytes.

The urinary biomarker data, adjusted for creatinine excretion, indicate that within the first 24 hours following an ETS nicotine exposure, only nicotine yields a significant correlation. Further, neither nicotine nor cotinine in the second (24 to 48-hr) urine sample related to the deliberate ETS nicotine exposure. The variability of these data is a consequence of inter-individual metabolic variation and *ad hoc* exposures observed in about 67% of the subjects. Many studies of urinary and/or salivary cotinine indicate that smokers may be discriminated from nonsmokers with high specificity. Discrimination of nonsmokers exposed to nicotine,

presumably from ETS, from those nonsmokers who claim no ETS exposure is more difficult and less specific.

Under the controlled conditions of this study, salivary nicotine is the most sensitive measure of acute nicotine intake (1-2 hrs) and salivary cotinine is such for more extended time periods due to its longer half-life in the body. To indicate nicotine exposure *only*, either urinary nicotine or cotinine is sufficient. Smokers may be readily discriminated from nonsmokers by biomarkers from either body fluid. Our data indicate that the intensity (dose), extent (duration), and timing of nicotine exposure in field settings may not be determinable by use of biomarker concentrations in any body fluid.

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